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The Effect of Biofield Energy Treated DMEM on the ALP Level in MG-63 cells: Bone-Health Biomarker

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The study was initiated to investigate the effect of Biofield Treatment on DMEM medium for bone health in MG-63 cells. The test item, DMEM medium was divided into two parts. The test item received Biofield Treatment by a renowned Biofield Energy Healer, Dahryn Trivedi and was labeled as the Biofield Treated DMEM, while the other part did not receive any treatment and denoted as untreated DMEM group. MTT assay showed that cell viability of the test item was more than 77%, which indicate a safe and non-toxic profile of the test items in MG-63 cells. The level of ALP was significantly ($p \leq 0.001$) increased by 42.86% in Biofield Treated DMEM compared with untreated DMEM. Overall, data envisaged that the Biofield Treated DMEM would play an important role for the growth of MG-63 cells along with an improved bone health parameter *i.e.* ALP level, which plays vital role in the promotion and maintenance of strong and healthy bones. Further, it could be used in numerous bone-related disorders *viz.* rickets, osteoporosis, deformed bones, Paget's disease of bone, bone loss and fractures, osteomalacia, bone and/or joint pain, osteoma, hormonal imbalance, stress, and aging.

Keywords: Biofield Energy, ALP, Osteoporosis, Osteosarcoma Cells, DMEM, Bone Health

Abbreviations

CAM: Complementary and Alternative Medicine, NCCAM: National Center for Complementary and Alternative Medicine; MG-63: Human Bone Osteosarcoma Cells, ALP: Alkaline phosphatase, DMEM: Dulbecco's Modified Eagle's Medium, FBS: Fetal Bovine Serum.

1. INTRODUCTION

Alkaline phosphatases (ALP) are the plasma membrane-bound glycoproteins, which are widely distributed in nature. Bone ALP is a useful biochemical marker of bone formation and is important for the bone mineralization also. Besides, ALP many other bone health biomarkers are available such as collagen, bone mineralization, calcium level, etc. However, ALP is one of the

important factors in bone health, which is widely used in bone health cell line studies. ALP is a phenotypic marker for maturation of osteoblasts and it also increases the local concentration of inorganic phosphate that is required for bone mineralization (Iba et al. 2014). Bone is a remarkable tissue with a strong functional structure to withstand intense physical activity that allows efficient movement. The skeleton system is one of the major physiological metabolic system (Lorincz et al. 2009). Skeleton system has dynamic tissue, which protects damage in various vital organs with mechanical forces. It acts as levers that transmit the mechanical force from one area of the body to another through muscle-driven motion. Metabolically, the skeleton system contained abundant blood cell types and is considered as the major calcium reservoir of the body. Bone health major nutrients such as calcium and vitamin D₃ play a vital role in preserving a healthy mineralized skeleton (Holick MF, 1996). Other rich sources are milk and dairy products, green and yellow vegetables, soybeans, and fish are also good sources of calcium for bone health. Besides, foods for specified health use (FOSHO) comprising calcium or other ingredients to improve calcium absorption can be utilized for increasing calcium intake. Calcium and vitamin D₃ have significant roles as anti-inflammatory, anti-arthritis, anti-osteoporosis, anti-stress, anti-aging, anti-apoptotic, wound healing, anti-cancer, anti-psychotic, and anti-fibrotic roles (Flynn A, 2003; Cashman KD, 2007; Lips P, 2001). MG-63 cells derived from juxtacortical osteosarcoma. The response of MG-63 cells to 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) administration has been studied to be similar to normal human osteoblast cells (Czekanska et al. 2012). Hence, MG-63 cell line is widely used for studying the potential of any test compounds to improve the bone health (Luo and Liao, 2003). Authors evaluated the *in vitro* effect of the Biofield Energy Treated DMEM media as a test item on bone health using MG-63 cells for ALP biomarker.

Within the growing demand for CAM therapies, Biofield or energy medicine is emerging with significant benefits in various scientific fields. CAM therapies has extensively used in the community which include external qigong, Johrei, Reiki, therapeutic touch, yoga, Qi Gong, pranic healing, polarity therapy, Ayurvedic medicine, Tai Chi, deep breathing, guided imagery, chiropractic/osteopathic manipulation, meditation, massage, homeopathy, hypnotherapy, progressive relaxation, acupressure, mindfulness, acupuncture, special diets, relaxation techniques, Rolfing structural integration, healing touch, pilates, movement therapy, traditional Chinese herbs and medicines in biological systems both *in vitro* and *in vivo* (Rubik B, 2012). Biofield contains putative bioenergy, which is channeled by a renowned practitioner from a distance. Biofield Energy Healing as a CAM showed significant results in biological studies (Barnes et al. 2008). The National Center for Complementary and Alternative Medicine (NCCAM) recommended that Biofield Therapies in the subcategory of Energy Therapies (Frass et al. 2012). The Trivedi Effect[®] has been reported with significant revolution in the physicochemical properties of polymers, metals, and ceramics (Trivedi and Tallapragada, 2008; ^{a,b}Trivedi, 2015), improved agricultural yield and quality (^{c,d}Trivedi, 2015), transformed antimicrobial characteristics (^{e,f,g}Trivedi, 2015), biotechnology (^hTrivedi, 2015; Nayak and Altekar 2015), improved bioavailability (^{a,b,c}Branton and Jana, 2017), skin health (Kinney et al. 2017; Singh et al. 2017), nutraceuticals (^{a,b}Trivedi, 2017), cancer research (^{l,j}Trivedi, 2015) human health and wellness (Anagnos et al. 2018; Lee et al. 2018; Stutheit et al. 2018). In this context, authors planned to evaluate the effect of Biofield Treatment on bone health in MG-63 cells using DMEM medium as a test item.

2. MATERIAL AND METHODS

2.1. Requirement of Chemicals

Rutin hydrate was purchased from TCI, Japan. Fetal bovine serum (FBS) and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Life Technology, USA. Antibiotics solution (penicillin-streptomycin) was procured from HiMedia, India, while 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium) (MTT), Direct Red 80, and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma, USA. All the other chemicals used in this experiment were analytical grade procured from India.

2.2. Cell Culture

Human bone osteosarcoma cell line -MG-63 was used as test system in the present study. The MG-63 cell line was maintained in DMEM growth medium for routine culture supplemented with 10% FBS. Growth conditions were maintained as 37°C, 5%CO₂ and 95% humidity and subcultured by trypsinisation followed by splitting the cell suspension into fresh flasks and supplementing with fresh cell growth medium. Three days before the start of the experiment (*i.e.*, day -3), the growth medium of near-confluent cells was replaced with fresh phenol-free DMEM, supplemented with 10% charcoal-dextran stripped FBS (CD-FBS) and 1% penicillin-streptomycin (Trivedi et al. 2018).

2.3. Experimental Design

The experimental groups consisted of group 1 (G-I) with cells in untreated DMEM. Group 2 (G-II) consisted of positive control (rutin hydrate) at non-cytotoxic concentrations. Further, group 3 (G-III) included the test item group, which was Biofield Treated DMEM medium.

2.4. Consciousness Energy Healing Treatment Strategies

The test item, DMEM medium was divided into two parts. One part each of the test item was treated with the Biofield Energy by a renowned Biofield Energy Healer, Dahryn Trivedi and coded as the Biofield Energy Treated DMEM, while the second part did not receive any sort of treatment and referred as the untreated DMEM group. This Biofield Energy Healing Treatment was provided by Dahryn Trivedi remotely for ~5 minutes. Biofield Energy Healer was remotely located in the USA, while the test item was located in the research laboratory of Dabur Research Foundation, New Delhi, India. This Biofield Energy Treatment was administered for 5 minutes through the Healer's unique Energy Transmission process remotely to the test sample under laboratory conditions. Dahryn Trivedi in this study never visited the laboratory in person, nor had any contact with the test item (DMEM). Further, the control group was treated with a "sham" healer for comparative purposes. The "sham" healer did not have any knowledge about the Biofield Energy

Treatment. After that, the Biofield Energy Treated and untreated samples were kept in similar sealed conditions for experimental study.

2.5. MTT Assay for the Assessment of Non-cytotoxic Concentration

For the evaluation on non-cytotoxic concentration of the test items (untreated and Biofield Treated DMEM) the MTT cell viability assay was performed in human bone osteosarcoma cell line (MG-63) as per Trivedi et al. 2018 (Czekanska et al. 2012). The percentage cytotoxicity of the test items were calculated with the help of Equation (1):

$$\% \text{ Cytotoxicity} = (1 - X/R) * 100 \dots \dots \dots (1)$$

Where, X = Absorbance of treated cells; R = Absorbance of untreated cells

The percentage cell viability corresponding to each treatment was calculated with the help of Equation (2):

$$\% \text{ Cell Viability} = (100 - \% \text{ Cytotoxicity}) \dots \dots \dots (2)$$

The concentrations $\geq 70\%$ cell viability was considered as safe and non-toxic.

2.6. Alkaline Phosphatase (ALP)

Evaluation of alkaline phosphatase (ALP) activity of the untreated and Biofield Treated DMEM in human bone osteosarcoma cell line (MG-63) was conducted as per Trivedi et al. 2018 (Stutheit et al. 2018; Trivedi et al. 2018). The level of ALP enzyme was recorded as mg/mL with respect to the untreated DMEM group.

2.7. Statistical Analysis

One-way analysis of variance (ANOVA) was used for multiple group comparison followed by post-hoc analysis by Dunnett's test. Statistically significant values were set at the level of $p \leq 0.05$. Data were represented as mean \pm standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1. MTT Assay for the Assessment of Non-cytotoxic Concentration

For the determination of non-cytotoxic concentration of the test items (untreated and Biofield Energy Treated DMEM) by MTT cell viability assay is shown in Figure 1. The results showed that the test sample was found to have significant cell viability with more than 80%. Moreover, the positive control (rutin) showed more than 77% cell viability (Figure 1). Hence, MTT data suggested that the untreated and Biofield Energy Treated DMEM were found as safe in MG-63 cells. Thus, the Biofield Energy Treated DMEM was used further to study the bone health parameter, alkaline phosphatase (ALP) activity in MG-63 cells.

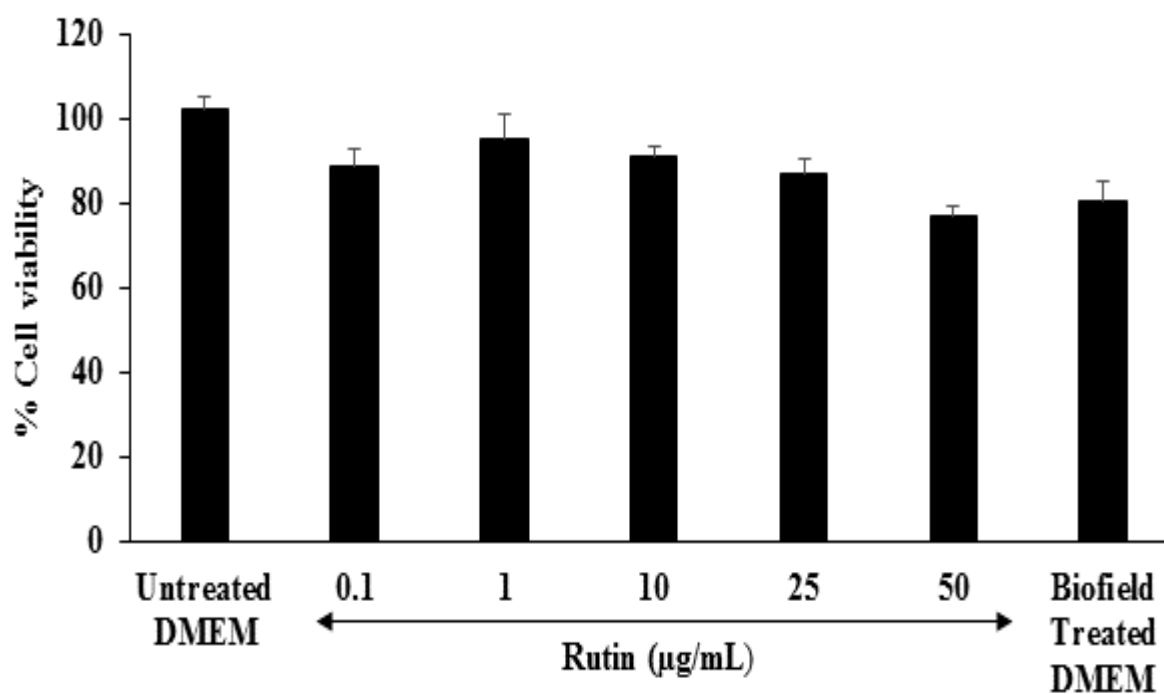


Figure 1: Determination of non-cytotoxic concentration using MTT cell viability assay of the positive control (rutin) and test items (DMEM) on MG-63 cell line.

3.2. Alkaline Phosphatase (ALP) Enzyme Activity

Alkaline Phosphatase (ALP) enzyme is the member of a family of zinc metalloprotein enzymes, which reflects the biosynthetic activity of these bone-forming cells. Bone ALP is considered as one of the sensitive and reliable indicator of bone metabolism activity. However, alteration in bone ALP results in serious bone health disorders such as post-menopausal women, bone cancers, osteoporosis, and healing fracture, Paget's disease of bone, acromegaly, bone growth, osteogenic sarcoma, myelofibrosis, leukemia, or bone metastases, and rarely myeloma. The level of bone ALP can be overcome with help of various nutraceutical supplements or vitamin D₃, calcium, etc. (Anonymous; Jesudason et al. 2002; Seeman, 2009). Biofield Energy Treated DMEM was tested with respect to the results of ALP enzyme level (mg/mL) on MG-63 cells and the data are shown in Figure 2. The positive control, rutin showed a significantly ($p \leq 0.001$) increased value of ALP by 214.29%, 228.57%, and 314.29% at 0.01, 0.1, and 1 $\mu\text{g/mL}$, respectively with respect to the untreated DMEM group. The Biofield Energy Treated DMEM showed a significant ($p \leq 0.001$) increase in the ALP level by 42.86% as compared with the untreated DMEM group (Figure 2).

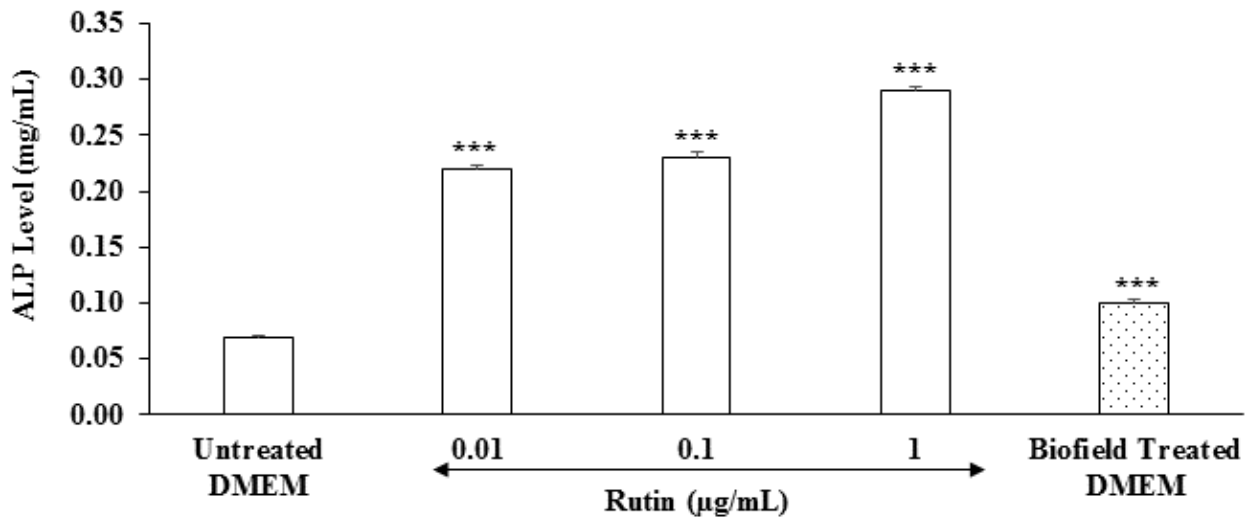


Figure 2: The potential effect of the test items (untreated and Biofield Treated DMEM) on the level of alkaline phosphatase (ALP) in MG-63 cells. *** $p \leq 0.001$ vs. untreated DMEM.

Thus, overall the bone health experimental data concluded that the Biofield Energy Healing Treatment in the DMEM media showed a significant improved level of the bone ALP as compared with the untreated DMEM group, which could be the best supplementation to treat various bone and age-related diseases such as osteoporosis (Golub, 2007). Overall, the data suggested that Consciousness Energy Treated DMEM could be used to improve the level of ALP.

4. CONCLUSIONS

The *in vitro* effect of Biofield Energy Healing based DMEM on bone health was studied and results showed significant improvement with respect to ALP activity. MTT assay for cell viability showed a significant improved cell viability with more than 77% among all the tested groups, which suggested that the test sample are found safe and nontoxic. Further, the bone health parameter, the level of ALP was increased significantly by 42.86% in Biofield Treated test item group compared with untreated DMEM. Therefore, Biofield Treated DMEM was found to have a significant impact on bone ALP level, which are very vital to combat various bone disorders. The Consciousness Energy Healing based DMEM might be a suitable alternative media for cell growth and for the management of various bone-related disorders *viz.* Paget's disease of bone, osteoporosis, rickets, osteomalacia, deformed bones, bone and/or joint pain, increased frequency of fractures, osteoma, hormonal imbalance, stress, bone loss and fractures, aging, and other bone diseases that are caused by problems with the rate of bone growth, poor nutrition, or genetics. On the other hand, it might be useful to improve normal cell growth, communication, cycling, proliferation, differentiation, and neurotransmission. Further, it can also be utilized in organ transplants (for example kidney transplants, liver transplants and heart transplants), hormonal imbalance, aging, and various immune related disease conditions such as Ulcerative Colitis,

Hashimoto Thyroiditis, Alzheimer's Disease, Dermatitis, stress, Irritable Bowel Syndrome, Systemic Lupus Erythematosus, Sjogren Syndrome, Asthma, Pernicious Anemia, Multiple Sclerosis, Atherosclerosis, Myasthenia Gravis, Aplastic Anemia, Diverticulitis, Hepatitis, Graves' Disease, Dermatomyositis, Diabetes, Parkinson's Disease, etc.

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